P20825.P01

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Form PTO-1390	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER P20825
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/787437

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP99/00388	29 January 1999	30 September 1998

TITLE OF INVENTION

APOPTOSIS INDUCING AGENT

APPLICANT(S) FOR DO/EO/US

Yoko AIDA and Masakazu KAMATA

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. _X_ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ___ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. _X_ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. _X_ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. _X_ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. _X_ is attached hereto (required only if not communicated by the International Bureau).
   b. _X_ has been communicated by the International Bureau.
   c. ___ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. _X_ An English language translation of the International Application as filed (35 U.S.C. 371 (c)(2)).
7. ___ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ___ are attached hereto (required only if not communicated by the International Bureau).
   b. ___ have been communicated by the International Bureau.
   c. ___ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ___ have not been made and will not be made.
8. ___ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3))
9. _X_ An oath or declaration of the inventor(s) (35 U.S.C. .371(c)(4)).
   "Unexecuted"
10. ___ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (U.S.C. 371(c)(5)).

Items 11 to 16 below concern other document(s) or information included:

11. Assignee: RIKEN of Saitama, JAPAN
12. ___ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
13. ___ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
14. _X_ A FIRST preliminary amendment.
    ___ A SECOND or SUBSEQUENT preliminary amendment.
15. ___ A substitute specification.
16. ___ A change of power of attorney and/or address letter.
17. ___ Figure of Drawing to be published_____
18. _X_ Other items or information:
    Cover Sheet and International Application as published in Japanese.
    PCT/RO/101-PCT Request.
    PCT/IPEA/408(in Japanese).
    PCT/IPEA/409.
    PCT/IB/301.
    PCT/IB/304.
    PCT/IB/308.
    PCT/IB/332.
    PCT/IB/338.
    PCT/ISA/210(in Japanese and English).
    Cover Letter under 35 USC 371 and 1.495.
    Claim of Priority.

U.S. APPLICATION NO. (If known, see 37 C 1.5) 09/787437	INTERNATIONAL APPLICATION NO. PCT/JP99/00388	ATTORNEY'S DOCKET NUMBER P20825

19. ___ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Search report has been prepared by the EPO or JPO. . . . . $ 860.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482). . . . . $ 690.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO(37 CFR 1.445(a)(2). . . . . $ 710.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO. $1,000.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4). . . . . $ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ___ 20 ___ 30 months from the earliest claimed priority date (37 CFR 1.492(c)).	$	

Claims	Number Filed	Number Extra	RATE		
Total Claims	21 - 20 =	1	X $18.00	$18.00	
Independent Claims	2 - 3 =	0	X $80.00	$0.00	
Multiple dependent claim(s) (if applicable)			+ $270.00	$0.00	
			TOTAL OF ABOVE CALCULATIONS =	$878.00	
___ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by ½.				$	
			SUBTOTAL =	$878.00	
Processing fee of $130.00 for furnishing the English translation later than ___ 20 ___ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).			+		
Extension of Time fee in the amount of $					
			TOTAL NATIONAL FEE =	$878.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property			+		
			TOTAL FEES ENCLOSED =	$878.00	
			Amount to be refunded	$	
			Charged	$	

a. _X_ A check in the amount of $878.00 to cover the above fees is enclosed.

b. ___ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees.

c. _X_ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0089.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO **CUSTOMER NO. 7055**
AT THE PRESENT ADDRESS OF:
Bruce H. Bernstein
GREENBLUM & BERNSTEIN, P.L.C.
1941 Roland Clarke Place
Reston, VA 20191
(703) 716-1191

Leslie Morgan Reg. No. 33,329
SIGNATURE
Bruce H. Bernstein
NAME

29,027
REGISTRATION NUMBER

P20825.A01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Yoko AIDA et al.

Serial No : Not Yet Assigned (National Stage of PCT/JP99/00388)

Filed : Concurrently Herewith (International Filing Date January 29, 1999)

For : APOPTOSIS INDUCING AGENT

PRELIMINARY AMENDMENT

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to calculation of the filing fees and the examination of the above-identified patent application on the merits, the Examiner is respectfully requested to amend the claims as follows:

IN THE CLAIMS

Please amend the claims as follows (a marked-up copy of the claim amendments is provided as an attachment to this Amendment):

4. (Amended-Clean Text) An apoptosis-inducing gene encoding the protein according to claim 1.

8. (Amended-Clean Text) A medicament comprising the protein according to claim 1 as an active ingredient.

12. (Amended-Clean Text) A method for preventive and/or therapeutic treatment of a cancer or AIDS, which comprises the step of administering a therapeutically effective amount of the protein according to claim 1.

Please add new claims 14-21 as follows:

14. ---An apoptosis-inducing gene encoding the protein according to claim 2.

15. An apoptosis-inducing gene encoding the protein according to claim 3.

16. A medicament comprising the protein according to claim 2 as an active ingredient.

17. A medicament comprising the protein according to claim 3 as an active ingredient.

18. The medicament according to claim 16, which is used for preventive and/or therapeutic treatment of a cancer or AIDS.

19. The medicament according to claim 17, which is used for preventive and/or therapeutic treatment of a cancer or AIDS.

20. A method for preventive and/or therapeutic treatment of a cancer or AIDS, which comprises the step of administering a therapeutically effective amount of the protein according to claim 2.

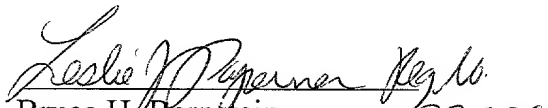
21. A method for preventive and/or therapeutic treatment of a cancer or AIDS, which comprises the step of administering a therapeutically effective amount of the protein according to claim 3.---

REMARKS

By the above amendment, claims 4, 8, and 12 have been amended and claims 14-21 have been added to delete multiple dependency.

If there should be any questions, the Examiner is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,
Yoko AIDA et al.



Bruce H. Bernstein
Reg. No. 29,027

33,329

March 26, 2001
GREENBLUM & BERNSTEIN, P.L.C.
1941 Roland Clarke Place
Reston, VA 20191
(703) 716-1191

MARKED-UP COPY OF AMENDED CLAIMS

4. (Amended) An apoptosis-inducing gene encoding the protein according to claim 1 [any one of claims 1 to 3].

8. (Amended) A medicament comprising the protein according to claim 1 [any one of claims 1 to 3] as an active ingredient.

12. (Amended) A method for preventive and/or therapeutic treatment of a cancer or AIDS, which comprises the step of administering a therapeutically effective amount of the protein according to claim 1 [any one of claims 1 to 3].

SPECIFICATION

APOPTOSIS INDUCING AGENT

Technical Field

The present invention relates to a protein having an apoptosis-inducing action and a gene encoding said protein.

BACKGROUND ART

Apoptosis is cell death that occurs under various physiological conditions, which differs from necrosis occurring due to physical injuries, chemical toxicants and so forth (Kerr, J.F. and Wyllie, A.H., Br. J. Cancer, 26, pp.239-257, 1972), and is also referred to as programmed cell death. Apoptosis is induced by cell damages by cytotoxic T cells, radiation irradiation, cytokines such as tumor necrosis factor (TNF), anti-CD3 antibodies and so forth, and apoptosis is also observed in spontaneous regression of malignant tumors. It is expected that gene therapies and destruction of specific cancer cells will become possible by using a gene or a gene product exogenously inducing cell apoptosis.

Human immunodeficiency virus type 1 (HIV-1), the causative virus of human acquired immunodeficiency syndrome (AIDS), has accessory genes (nef, vpr, vpu, and vif) which are not essential for its own replication in addition to the structural genes and regulatory genes. A gene product of vpr (protein Vpr), one of the accessory genes, has been focused as a key factor for the onset of AIDS since, for example, it increases virus infection efficiency and triggers production of viruses from latent HIV infected cells. Moreover, it has also been elucidated that the protein Vpr has a wide variety of physiological actions such as inhibition of cell growth, induction of differentiation, induction of apoptosis, inhibition of apoptosis and induction of nucleus polyploidization.

Disclosure of the Invention

An object of the present invention is to provide a gene having apoptosis-inducing action and a gene product thereof. The inventors of the present invention conducted various studies to achieve the aforementioned object. As a result,

they found that a mutant, in which 15 amino acid residues were deleted from the carboxyl terminal of Vpr protein consisting of 96 amino acid residues encoded by vpr as the accessory gene of HIV-1, had extremely high apoptosis-inducing activity, and thereby exhibited suppressing activity on cell proliferation. The present invention was achieved on the basis of these findings.

The present invention thus provides an apoptosis inducing agent which comprises a protein as being Vpr protein encoded by vpr gene of HIV-1 in which 15 amino acid residues from C-terminal are deleted. From another aspect of the present invention, there is provided an apoptosis inducing agent which comprises a protein having the amino acid sequence of the aforementioned protein wherein one to several amino acids are substituted, inserted, and/or deleted, and having apoptosis-inducing activity. The present invention also provides an apoptosis-inducing gene encoding the aforementioned protein.

From further aspects of the present invention, there are provided a method for inducing cell apoptosis by using the aforementioned protein or the aforementioned gene; a recombinant vector comprising the aforementioned gene; and a method for inducing cell apoptosis by using the aforementioned recombinant vector.

As a still further aspect of the present invention, there is provided a medicament comprising the aforementioned protein as an active ingredient. The medicament comprising said protein as an active ingredient is useful as, for example, a anticancer agent or anti-AIDS agent. There is also provided a medicament comprising the recombinant vector as an active ingredient. The medicament can be used for a gene therapy of a cancer or AIDS. The present invention further provides a method for treating a cancer or AIDS which comprises the step of administering an effective amount of the aforementioned protein or the aforementioned recombinant vector to a patient, and use of the aforementioned protein or the aforementioned gene for a manufacture of the aforementioned medicament.

Best Mode for Carrying out the Invention

The protein of the present invention is composed of 81 amino acid residues, which is Vpr protein encoded by vpr gene of HIV-1 in which 15 amino acid residues from the C-terminal are deleted (hereafter, this protein is also referred to as "C81 mutant protein" in the specification). This C81 mutant protein can be easily prepared

according to the method described in the example mentioned below. The C81 mutant protein can also be prepared by utilizing the nucleic acid sequences of the vpr gene of HIV-1 or the amino acid sequence of the Vpr protein (Adachi, A. et al., J. Virol., 59, pp. 284-291, 1986).

The C81 mutant protein of the present invention is characterized by markedly increased apoptosis-inducing action compared to the Vpr protein. The increased apoptosis-inducing action of the C81 mutant protein of the present invention can easily be determined by those skilled in the art according to the method of the example in the specification. In addition, the protein of the present invention is characterized to have substantially no ability to arrest cells in the G_2 phase, unlike the Vpr protein.

A protein which has the amino acid sequence of the aforementioned C81 mutant protein wherein one to several amino acid residues are substituted, inserted, and/or deleted and has apoptosis-inducing action similar to that of the C81 mutant protein also falls within the scope of the present invention (hereinafter referred to as a "modified protein"). Both DNA and RNA sequences comprising a nucleic acid sequence coding for the C81 mutant protein or a modified protein fall within the scope of the gene of the present invention. The genes can be easily obtained according to the method described in the aforementioned reference.

The aforementioned modified protein can be prepared by subjecting *Escherichia Coli* or the like having a DNA encoding the amino acid sequence of the C81 mutant protein to a treatment for mutation by using an agent such as N-nitro-N'-nitro-N-nitrosoguanidine, collecting a gene encoding a modified protein from the microbial cells, and then performing conventional procedure for gene expression. It is also possible to directly introduce deletion, substitution, or addition of nucleotides into the aforementioned gene by directly treating the gene with an agent such as sodium sulfite, or applying site-directed mutagenesis (Kramer, W. et al., Methods in Enzymology, 154, p. 350, 1987), recombinant PCR method (PCR Technology, Stockton press, 1989) or the like.

The protein of the present invention is useful as an apoptosis inducing agent. For example, the protein can be used as a medicament for inducing apoptosis in cancer cells to kill the cells. The protein is also useful for elimination of latent infected cells with human immunodeficiency virus (HIV) and development of techniques for the elimination. Therefore, the medicament comprising the protein of the present

invention can be used for preventive and/or therapeutic treatment of a cancer or preventive and/or therapeutic treatment of acquired immunodeficiency syndrome (AIDS). A method for administration, a dose, a dosage form and so forth of the medicament of the present invention can be appropriately selected by those skilled in the art and are not particularly limited.

For the purpose of utilization as the aforementioned medicament, the protein of the present invention may be fused with other polypeptide. Fusion proteins containing the amino acid sequence of the protein of the present invention as a partial sequence and genes coding for such fusion proteins also fall within the scope of the present invention. For example, it becomes possible to specifically induce apoptosis in target cells such as cancer cells by preparing a protein fused with a monoclonal antibody or a fragment thereof specifically recognizing the target cells. The protein of the present invention is also expected to be useful in treatment of diseases associated with apoptosis resistance, and is useful as a reagent in the fields of biochemistry, genetic engineering and so forth.

The gene of the present invention is useful for preparing the protein of the present invention, as well as for gene therapy of diseases associated with apoptosis resistance. For example, the gene can be used for gene therapy for preventive and/or therapeutic treatment of a cancer or AIDS. Procedures for the gene therapy are not particularly limited, and in general, the gene of the present invention is inserted into a vector, and then the recombinant vector is introduced into a living body for expression of the gene of the present invention. Various vectors for introducing the gene into living bodies are known, and those skilled in the art can chose an appropriate vector. Techniques for regulating expression of a gene in a specific cell are also available for those skilled in the art. In addition, HIV infected cells can be directly and specifically destroyed by ligating the gene of the present invention to a region downstream from HIV-1 LTR, and then introducing the resulting gene into a living body after the gene is encapsulate in liposomes modified with anti-HIV gp120 antibody.

Example

The present invention will be more specifically explained with reference to the following example. However, the scope of the present invention is not limited to the following example.

1. Materials and methods

A Flag sequence was ligated to the 5' end of vpr gene fragment of HIV-1 infectious DNA clone pNL432 and inserted into high-level expression vector pME18neo. This procedure will be explained below.

(1) Primers designed for amplifying a gene coding for the C81 mutant protein (hereafter, referred to as "C81 mutant gene") were as follows:

Sense Primer: 5'-GAAGATATCCGAACAAGCCCCAGAAGAC-3'

Anti-sense Primer: 5'-GGTCTAGATCATATTCTGCTATGTCGACAC-3'

In addition to a cohesive sequence, an EcoRV site for ligation of Flag-Tag was added to the 5' end of the sense primer, and an XbaI site for ligation of subcloning vector was added to the 3' end of the anti-sense primer (restriction sites are underlined). PCR was performed by using these primers and using an infectious DNA clone pNL432 of HIV-1 isolate NL43 (Adachi, A. et al., J. Virol., 59, pp. 284-291, 1986) as a template to amplify the C81 mutant gene fragment.

(2) After heat denaturation in a reaction solution containing 1 μg of the template DNA, PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin), 0.2 mM dNTP, 50 pmol each of the primers, and 2.5 units of Ampli Taq polymerase (Perkin Elmer Cetus) at 94°C for 5 minutes, amplification was performed by 35 cycles of reaction at 94°C for 1 minute, at 54°C for 1 minute, and at 72°C for 2 minutes. Then, extension reaction was performed at 72°C for 10 minutes. The resulting PCR product was treated with EcoRV and XbaI for 4 hours or more, and the resulting DNA was fractioned by agarose gel electrophoresis. Then, the target DNA fragment was eluted and purified by using a GENECLEAN II KIT.

(3) Fro ligation of the amplified fragment and the Flag-Tag sequence, the vpr gene fragment amplified by PCR was then ligated to pBluescript SK+-II vector ligated beforehand with Flag-Tag and treated with EcoRV and XbaI, and transformed into *Escherichia Coli* competent cells XLI-Blue. Then, a DNA fragment of the C81 mutant gene was excised from the Fvpr/pBluescript SK+-II at the NotI and XhoI cleavage sites. The target DNA fragment was eluted by using the GENECLEAN II KIT, ligated to pME18Neo, and then introduced into XLI-Blue to obtain transformed cells. The plasmid DNA was prepared by the SDS method and purified by the cesium chloride equilibrium density gradient centrifugation method.

Each of the above plasmid, wild-type vector, and a control vector was introduced into HeLa cells by electroporation. Effect on cell growth was analyzed by the colony formation method. Twelve hours after the introduction, 5×10^5 cells were placed in a 10-cm petri dish and cultured in a selective medium containing G418 for 12 days. After fixation with methanol, giemsa staining was performed and the number of colonies was counted. At this time, introduction efficiency was calculated for each mutant by the β-Gal staining and the number of colonies was corrected. The cell cycle was analyzed by flow cytometry.

The C81 mutant gene expression plasmid was transiently co-introduced with the GFP expression plasmid. After 48 hours, the cell was fixed by using 1% formamide/PBS and then 70% methanol, stained with a PI staining solution and analyzed by FACS. Cells introduced with C81 mutant gene and non-introduced cells were distinguished by using fluorescence of GFP as a marker, and the DNA content in each fraction was examined. The cells were classified into + those having ability to arrest cells in G_2 comparable to the wild-type; ± weaker than the wild-type, and − no ability. Similarly, 48 hours after the introduction, the cells were stained by dual fluorescent staining using anti-Flag antibodies or anti-minichromosome maintenance (MCM) antibodies and then investigated under a confocal laser microscope. MCM negative cells were determined as cells in the G_2 phase.

To detect growing cells, the cells were cultured in the presence of bromodeoxyuridine (BrdU) for 30 minutes and then subjected to fluorescent staining using anti-BrdU antibodies. Further, 48 hours after the introduction, the cells were subjected to fluorescent staining by using biotin-labeled annexin V and PE-labeled streptavidin. The cells were observed under a confocal laser microscope by using GFP positive cells as a marker of the cell introduced with C81 mutant gene. Annexin V positive cells were determined as apoptosis-induced cells.

2. Results

In the HeLa cells introduced with the C81 mutant gene encoding the C81 mutant protein, which corresponded to the Vpr protein with deletion of 15 amino acid residues from the C-terminal, the ability of forming colonies was reduced about 30% compared to the cells introduced with the control vector, even though the G_2-phase arrest was not observed. The uptake of BrdU by the cells introduced with the vpr

having the C-terminal deletion was also markedly reduced compared to the cells introduced with the control vector. Further, fluorescent staining of the cells using anti-serum for MCM as the G_2-phase marker revealed that the above suppressing action against cell proliferation was not induced by the G_2-phase arrest.

From the above results, it was verified that the cell proliferation was markedly suppressed in the cells introduced with the Vpr having the C-terminal deletion by a mechanism different from the G_2-phase arrest. For further investigation, the cells were stained with annexin V/biotin. As a result, remarkable and rapid increase of the proportion of cells under apoptosis was observed in the cells introduced with the Vpr having the C-terminal deletion compared to the cells introduced with the wild-type Vpr. Thus, it was revealed that the mutant Vpr protein having deletion of 15 amino acid residues at the carboxyl terminal had remarkably high apoptosis inducing activity.

Table 1

Examined Item	Type of cells introduced with expression vector		
	C81 mutant	Wild type	Control vector
G_2-phase arrest[1)]	−	+	−
Percentage of positive cells in fluorescent staining with anti-MCM antibody[2)]	63.6%	18.3%	80.0%
Colony forming ability[3)]	72.8%	7.6%	100.0%
Percentage of cells taking up BrdU[4)]	20.1%	19.5%	34.8%
Percentage of positive cells in staining with annexin V/biotin[5)]	20.7%	1.4%	1.3%

1) +: having ability of arresting cells in the G_2 phase comparable to the wild-type, ±: weaker than the wild-type, −: no ability of arresting cells in the G_2 phase.

2) MCM negative: cell in the G_2 phase

3) The introduction efficiency was calculated for each mutant by using β-Gal staining to correct the number of colonies. Values are indicated as % based on the number of colonies of the cells introduced with the control vector, which is taken as 100.

4) Anti-BrdU antibody positive: cells under proliferation

5) Annexin V positive: cells under apoptosis induction

What is claimed is:

1. An apoptosis inducing agent which comprises a protein as being Vpr protein encoded by vpr gene of HIV-1 wherein 15 amino acid residues from the C-terminal are deleted.

2. An apoptosis inducing agent which comprises a protein having amino acid sequence of the protein according to claim 1 wherein one to several amino acids are substituted, inserted, and/or deleted, and having apoptosis-inducing activity.

3. The apoptosis inducing agent according to claim 2, which comprises said protein having substantially no ability of arresting a cell in G_2 phase.

4. An apoptosis-inducing gene encoding the protein according to any one of claims 1 to 3.

5. A method for inducing apoptosis of a cell by using a protein as being Vpr protein encoded by vpr gene of HIV-1 wherein 15 amino acid residues from the C-terminal are deleted.

6. A recombinant vector containing the gene according to claim 4.

7. A method for inducing apoptosis of a cell by using the recombinant vector according to claim 6.

8. A medicament comprising the protein according to any one of claims 1 to 3 as an active ingredient.

9. The medicament according to claim 8, which is used for preventive and/or therapeutic treatment of a cancer or AIDS.

10. A medicament comprising the recombinant vector according to claim 6 as an active ingredient.

11. The medicament according to claim 10, which is used for preventive and/or therapeutic treatment of a cancer or AIDS.

12. A method for preventive and/or therapeutic treatment of a cancer or AIDS, which comprises the step of administering a therapeutically effective amount of the protein according to any one of claims 1 to 3.

13. A method for preventive and/or therapeutic treatment of a cancer or AIDS, which comprises the step of administering a therapeutically effective amount of the recombinant vector according to claim 6.

ABSTRACT

An apoptosis inducing agent which comprises a protein as being Vpr protein encoded by vpr gene of HIV-1 wherein 15 amino acid residues from the C-terminal are deleted, and an apoptosis inducing gene encoding said protein. The agent induces apoptosis of cells and therefore useful as a medicament for treatment of a cancer or AIDS.

Declaration and Power of Attorney For Utility or Design Patent Application
特許出願宣言書

Japanese Language Declaration

私は、下欄に氏名を記載した発明者として、以下のとおり宣言する：

私の住所、郵便の宛先および国籍は、下欄に氏名に続いて記載したとおりであり、

名称の発明に関し、請求の範囲に記載した特許を求める主題の本来の、最初にして唯一の発明者である(一人の氏名のみが下欄に記載されている場合)か、もしくは本来の、最初にして共同の発明者である(複数の氏名が下欄に記載されている場合)と信じ、

上記発明の明細書(下記の欄でX印がついていない場合は、本書に添付)は、

☐ _____年_____月_____日に提出され、

米国出願番号 _____ とし、

(該当する場合) _____年_____月_____日に訂正されました。又は、

特許協定条約国際出願番号 _____ とし、

(該当する場合) _____年_____月_____日に訂正されました。

私は、前記のとおり補正した請求の範囲を含む前記明細書の内容を検討し、理解したことを陳述する。

私は、連邦規則法典第37編第1条第56項に定義されるとおり、特許資格の有無について重要な情報を開示すべき義務があることを認めます。

私は合衆国法典第35部第119条(a－d)項又は第365条(b)項に基づく、下記の外国特許出願又は発明者証出願、或いは第365条(a)項に基づく、少なくても米国以外の1ケ国を指名したPCT国際出願の外国優先権を主張し、更に優先権の主張に係わる基礎出願の出願日前の出願日を有する外国特許出願、又は発明者証出願或るいはPCT国際出願を以下に"なし"の箱に印をつけることにより明記する：

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

APOPTOSIS INDUCING AGENT

the specification of which is attached hereto unless the following box is checked:

☒ was filed on January 29, 1999 as United States Application Number 09/787,437 and was amended on March 27, 2001 (if applicable) or,

PCT International Application Number PCT/JP99/00388 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority under Title 35, United States Code §119(a-d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States, listed below. I have also identified below, by checking the "No" box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed:

Prior foreign applications 先の外国出願 (Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願の年月日)	Priority claimed 優先権の主張 Yes あり	No なし
10/277361	Japan	September 30, 1998	☒	☐
			☐	☐

☐ その他の外国特許出願番号は別紙の追補優先権欄にて記載する。

☐ Additional foreign application numbers are listed on a supplemental priority sheet attached hereto.

Japanese Language Utility or Design Patent Application Declaration

私は、合衆国法典第35部第119条(e)項に基づく、下記の合衆国仮特許出願の利益を主張する。

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

(Application No.) (出願番号)	(Day/Month/Year Filed) 出願の年月日
(Application No.) (出願番号)	(Day/Month/Year Filed) 出願の年月日
(Application No.) (出願番号)	(Day/Month/Year Filed) 出願の年月日

☐ その他の合衆国仮特許出願番号は別紙の追補優先権欄にて記載する。

☐ Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.

私は、合衆国法典第35部第120条に基づく下記の合衆国特許出願、又は第365条(c)項に基づく合衆国を指名したPCT国際出願の利益を主張し、本願の請求の範囲各項に記載の主題が合衆国法典第35部第112条第1項規定の態様で、先の合衆国特許出願又はPCT国際出願に開示されていない限度において、先の出願の出願日と本願の国内出願日又はPCT国際出願日の間に有効となった連邦規則法典第37部第1章第56条に記載の特許要件に所要の情報を開示すべき義務を有することを認める。

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or §365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application No.) (出願番号)	(Day/Month/Year Filed) (出願の年月日)	(現況) (特許済み、係属中　放棄済み)	(Status) (patented, pending, abandoned)
(Application No.) (出願番号)	(Day/Month/Year Filed) (出願の年月日)	(現況) (特許済み、係属中　放棄済み)	(Status) (patented, pending, abandoned)

☐ その他の合衆国又は国際特許出願番号は別紙の追補優先権欄にて記載する。

☐ Additional U.S. or international application numbers are listed on a supplemental priority sheet attached hereto.

私は、ここに自己の知識にもとずいて行った陳述がすべて真実であり、自己の有する情報および信ずるところに従って行った陳述が真実であると信じ、さらに故意に虚偽の陳述等を行った場合、合衆国法典第18部第1001条により、罰金もしくは禁錮に処せられるか、またはこれらの刑が併科され、またかかる故意による虚偽による陳述が本願ないし本願に対して付与される特許の有効性を損なうことがあることを認識して、以上の陳述を行ったことを宣言する。

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

私、下記署名者は、ここに記載の米国弁護士または代理人に本出願に関し特許商標庁にて取られるいかなる行為に関して、同米国弁護士又は代理人が、私に直接連絡なしに私の外国弁護士或るいは法人代表者からの指示を受け取り、それに従うようここに委任する。この指示を出す者が変更の場合には、ここに記載の米国弁護士又は代理人にその旨通知される。

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from either his foreign patent agent or corporate representative, if any, as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

Case # P20825

Japanese Language Utility or Design Patent Application Declaration

委任状： 私は、下記発明者として、下記に明記された顧客番号を伴う以下の弁護士又は、代理人をここに選任し、本順の手続きを遂行すること並びにこれに関する一切の行為を特許商標庁に対して行うことを委任する。そして全ての通信はこの顧客番号宛に発送される。

顧客番号 7055

現在選任された弁護士は下記の通りである。

POWER OF ATTORNEY: As a named inventor, I hereby appoint the attorney(s) and/or agent(s) associated with the Customer Number provided below to prosecute this application and transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to that Customer Number:

CUSTOMER NUMBER 7055

The appointed attorneys presently include:

Neil F. Greenblum	Reg. No. 28,394	Stephen M. Roylance	Reg. No. 31,296
Bruce H. Bernstein	Reg. No. 29,027	William E. Lyddane	Reg. No. 41,568
James L. Rowland	Reg. No. 32,674	William Pieprz	Reg. No. ~~33,630~~
Arnold Turk	Reg. No. 33,094	Leslie J. Paperner	Reg. No. 33,329

Address: **GREENBLUM & BERNSTEIN, P.L.C.**
1941 ROLAND CLARKE PLACE
RESTON, VA 20191

直接電話連絡先：（名称および電話番号） Direct Telephone Calls to: (*name and telephone number*)

GREENBLUM & BERNSTEIN, P.L.C.
(703) 716-1191

唯一のまたは第一の発明者の氏名	1-00	Full name of sole or first inventor	Yoko AIDA
同発明者の署名	日付	Inventor's signature	Yoko Aida Date June 4, 2001
住所		Residence	Ibaraki, JPANA JPX
国籍		Citizenship	Japan
郵便の宛先		Post Office Address	3-105, Shareru Tsukuba Matsushiro, 21-2
			Matsushiro 4-chome, Tsukuba, Ibaraki 305-0035 Japan
第2の共同発明者の氏名（該当する場合）	2-00	Full name of second joint inventor, if any	Masakazu KAMATA
同第2共同発明者の署名	日付	Second Inventor's signature	Date June 4, 2001
住所		Residence	Ibaraki, JAPAN JPX
国籍		Citizenship	Japan
郵便の宛先		Post Office Address	203, Green hill side hiyama, 3-10-24, Koyadai
			Tsukuba, Ibaraki 305-0074 JAPAN

（第六またはそれ以降の共同発明者に対しても同様な情報および署名を提供すること。）

(Supply similar information and signature for third and subsequent joint inventors.)